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Phenolic profiling in the pulp and peel of nine plantain cultivars (*Musa* sp.)

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ABSTRACT

The present study investigated the phenolic profiles of the pulp and peel of nine plantain cultivars and compared them to those of two dessert bananas of commercial interest (Grand Nain and Gros Michel), alongside a newly created hybrid, resistant to black sigatoka disease (F568). Identification and quantification of phenolic compounds were performed by means of HPLC–ESI-HR-MS and HPLC-DAD. Hydroxycinnamic acids, particularly ferulic acid-hexoside with 4.4–85.1 μg/g of dry weight, dominated in the plantain pulp and showed a large diversity among cultivars. Flavonol glycosides were predominant in plantain peels, rutin (242.2–618.7 μg/g of dry weight) being the most abundant. A principal component analysis on the whole data revealed that the phenolic profiles of the hybrid, the dessert bananas and the pure plantains differed from each other. Plantain pulps and peels appeared as good sources of phenolics, which could be involved in the health benefits associated with their current applications.

1. Introduction

Phenolic compounds are secondary metabolites produced in plants through the phenylpropanoid pathway and encompass a wide range of chemical classes, including phenolic acids, flavonoids, stilbenes and lignans (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). They are basically involved in plant defence mechanisms and are also known to exert numerous health promoting effects. They act as antioxidants and modulators of enzyme expression and thereby contribute to the alleviation of a wide range of chronic diseases, such as cancer, diabetes, skin damages, allergies, atherosclerosis and viral infections (Huang & Shen, 2012; Liu, 2004). Furthermore, phenolic compounds are exploited in food protection against alterations by microorganisms or by lipid oxidations (Maqsood, Benjakul, & Shahidi, 2013). They are therefore involved in the formulation of many dietary supplements, food additives and drugs and their natural sources are of great interest.

Plantain bananas as well as the other parts of the *Musa* sp. plant, which include roots, pseudostems, stems, leaves and flowers have long been used in folk medicine in Africa, India, Asia and America. Recent investigations highlighted their antioxidant, anti-bacterial, anti-ulcerogenic, anti-hypertension, anti-diabetic and anti-cancer activities (Imam & Akter, 2011). Phenolic compounds are believed to be partly responsible for these properties (Jawla, Kumar, & Khan, 2012). However, only scarce information on the phenolic composition of plantain bananas is currently available.

Three types of *Musa* sp. fruits can be distinguished: dessert, plantain cooking and non-plantain cooking bananas. Plantain banana cultivars are generally separated into two main groups, namely French and Horn, according to the presence or the absence of the male bud at harvest, respectively. In addition, new elite hybrids, with improved disease resistance, are currently being developed and increase plantain banana diversity.

Bananas have been reported as an important source of phenolic compounds (Schieber, Stintzing, & Carle, 2001). Flavonoids, including flavones (apigenin), flavanones (naringenin), and flavonols

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(myricetin, kaempferol and quercetin), were found in the sap of diverse dessert banana species (Pothavorn et al., 2010). Anthocyanin pigments (petunidin, malvidin, pelargonidin, delphinidin, cyanidin and peonidin) were identified in wild banana bracts (Kitdamrongsont et al., 2008). Epigallocatechin was identified as the major constituent of condensed tannins in the pulps of diverse dessert and plantain banana cultivars (Uclés Santos, Bakry, & Brillouet, 2010) and a leucocyanidin (flavan-3,4-diol) was identified in plantain banana pulp as the active anti-ulcerogenic molecule (Lewis, Fields, & Shaw, 1999). To the best of our knowledge, the whole phenolic profile of plantain pulp and peel has never been investigated.

In this study, we characterized for the first time the phenolic profile of the pulp and peel of diverse plantain cultivars. Their chemical patterns were further compared to the ones of two dessert banana cultivars and two samples of one newly created hybrid collected in two different pedo-climatic environments. The use of the plantain phenolic extracts in the food and pharmaceutical industry and the involvement of the identified phenolics in plantain diversity was finally discussed.

2. Materials and methods

2.1. Chemicals

Kaempferol-rutinoside, isorhamnetin-rutinoside, ferulic acid, and caffeic acid standards were purchased from Sigma–Aldrich (St. Louis, MO). Rutin was obtained from ExtraSynthese (Genay, France). Acetone of analytical grade was obtained from VWR-Prolabo (Briare, France). Methanol, acetonitrile, formic acid of HPLC grade were supplied by Biosolve (Valkenswaard, The Netherlands).

2.2. Plant materials

Banana fruits harvested in 2011 were provided by the African Research Centre on Banana and Plantain (CARBAP, Njombe in Cameroon), which is hosting the world largest field *Musa* collection (Tomekpe, Kwa, Dzomeku, & Ganry, 2011). The collected fruits were composed of 8 plantain cultivars, including 2 French plantains (Red Yade and Mbeta 1) and 6 Horn plantains (Big Ebanga, Moto Ebanga, Batard, Essang, Mbouroukou N° 1, and Mbouroukou N° 3), 1 dessert banana (Gros Michel) and 2 samples of the same hybrid (F568 resistant to the black sigatoka disease) harvested at two different pedo-climatic environments: Njombe (an elevation of 80 m, a young volcanic soil, a mean temperature of 27 °C and an annual precipitation of 2600 mm) and Bansoa (an elevation of 1400 m, an old volcanic soil, a mean temperature of 19 °C and an annual precipitation of 1500 mm). The sampling was completed by a commercialized plantain from Colombia and the commercialized dessert banana Grand Nain, known as "Banane Chiquita", was purchased in local markets in Belgium. For each cultivar, 3 ripe fruits were treated separately. The peel and the pulp were separately frozen in liquid nitrogen and further freeze-dried, ground (particle size < 1 mm), and kept at −20 °C under nitrogen.

2.3. Identification of phenolic compounds of plantain pulp and peel

2.3.1. Extraction

Approximately 0.5 g of freeze-dried pulp or peel, were extracted with 10 ml of acetone:water:acetic acid (50:49:1; v:v:v) containing 0.2 mM of ascorbic acid. The mixture was vortexed for 1 min and the extraction was carried out in a water bath under agitation at 40 °C for 1 h. The extract was centrifuged at 4 °C, for 20 min at 5000 g and the supernatant was collected. The residue was extracted two more times. The supernatants were combined and evaporated to dryness with a rotary evaporator at 40 °C.

2.3.2. HPLC–ESI-HR-MS analysis

The dried extracts of the cultivar Red Yade (used as plantain reference) were solubilized in water and poured onto a pre-conditioned C18 SPE column. Then fractions were produced by stepwise elution with increasing ethanol concentrations (0%, 5%, 7%, 10%, 15%, 20%, 25%, 50%, 75%, and 100%) as described by Lai et al. (2013). Fractions were evaporated to dryness in a SpeedVac, resuspended in 1 ml (for peel) and 0.5 ml (for pulp) of methanol 50% in water, and filtered through a 0.45 μm syringe filter (Macherey–Nagel, Düren, Germany), before being analysed by high performance liquid chromatography (HPLC) negative electrospray ionisation high-resolution mass spectrometry (ESI-HR-MS).

HPLC–ESI-HR-MS analysis was performed as described by Lai et al. (2013), using a HPLC system connected to a LTQ-Orbitrap-XL mass spectrometer. Briefly, an aliquot of 20 μl of each fraction was injected onto a Waters XSelect CSH C18 column (100 × 3 mm; 2.5 μm particle size) equipped with a guard column of the same type (Milford, MA). The mobile phases A (water) and B (acetonitrile, all with 0.1% formic acid) were used under the following elution gradient: 0–10 min, 0–15% B; 10–25 min, 15% B; 25–30 min, 15–25% B; 30–35 min, 25–100% B; 35–40 min, 100% B; 40–45 min, 100–0% B; 45–50 min, 0% B, at a flow rate of 0.75 ml/min with a 50:50 flow-split just before the mass detector. All the operating parameters were the same as described by Lai et al. (2013) except that the tuning of the ESI source was done with rutin with the following ESI conditions: 5 V of spray voltage; sheath gas (N_2) flow rate of 18 and auxiliary gas (N_2) flow rate of 30 arbitrary units; temperature of the capillary set at 275 °C; capillary voltage of −43 V; tube lens of −143 V.

2.4. Quantification by HPLC-DAD analysis

For the quantification, the dried extracts were resuspended in 1 ml of 50% methanol in water and filtered. The HPLC-Diode Array Detector (DAD) system described by Lai et al. (2013) was used. Simultaneous monitoring was set at 280 nm for benzoic acids, 320 nm for hydroxycinnamic acids, and 350 nm for flavonols. Phenolic compounds were identified by their retention times and spectral data and were quantified using five-point calibration curves. Caffeic acid, ferulic acid, rutin, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside and myricetin were used for the calibration curves with the working ranges of 0.8–12.5 μg/ml for caffeic acid and ferulic acid, 3.1–50 μg/ml for kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside and 6.25–100 μg/ml for rutin and myricetin. The detection and quantification limits were 0.024 μg/ml and 0.08 μg/ml for caffeic acid, 0.007 μg/ml and 0.022 μg/ml for ferulic acid, 0.07 and 0.24 μg/ml for rutin, 0.11 and 038 μg/ml for kaempferol-3-*O*-rutinoside, 0.10 and 0.34 μg/ml for isorhamnetin-3-*O*-rutinoside and 0.16 and 0.54 μg/ml for myricetin. The recovery rates (mean ± SD, $n = 4$) were determined for the phenolic acid ferulic acid and for the flavonol rutin. The values obtained for ferulic acid were 82.6 ± 1.7% in the pulp and 92.6 ± 4.3% in the peel. Those obtained for rutin were 100.5 ± 3.6% in the pulp and 93.73 ± 4.5% in the peel.

2.5. Statistical analysis

Statistical analyses of the data were performed by JMP 9.0 Statistical Discovery software from SAS and SAS Entreprise Guide 4.3. Tukey's test was used for mean value comparisons. Principal component analysis was performed to compare the phenolic profiles of the different banana cultivars.

3. Result and discussion

3.1. Major phenolic compounds of plantain pulp and peel

Pulp and peel extracts of the Red Yade plantain were analyzed by HPLC-DAD-ESI-HR-MS. Their phenolic profiles are illustrated in Fig. 1 and the putative identifications of the compounds are presented in Table 1. The proposed identifications are based on both the UV–Vis spectra (indicating the different phenolic compound classes), mass spectra (including molecular formulas of the parent ions, the related fragments and the lost moieties) and comparison with previous literature and reference data from the PubChem Compound database (http://pubchem.ncbi.nlm.nih.gov). Molecular formulas were considered when the mass error was below 4 ppm. The classification of phenolic compounds according to their UV–Vis spectra is based on the one reported by Robards, Prenzler, Tucker, Swatsitang, and Glover (1999).

Hydroxycinnamic acids dominated the phenolic profile of the pulp (Fig. 1A), whereas flavonols were predominant in the peel (Fig. 1B) of the Red Yade plantain cultivar.

In the pulp, hydroxycinnamic acids presented a maximum UV–Vis absorbance (λ_{max}) within the range of 320 and 330 nm. Peaks 4 and 5 were the major hydroxycinnamic acids and exhibited molecular ions $[M-H]^-$ at m/z 355.1032 and m/z 385.1137

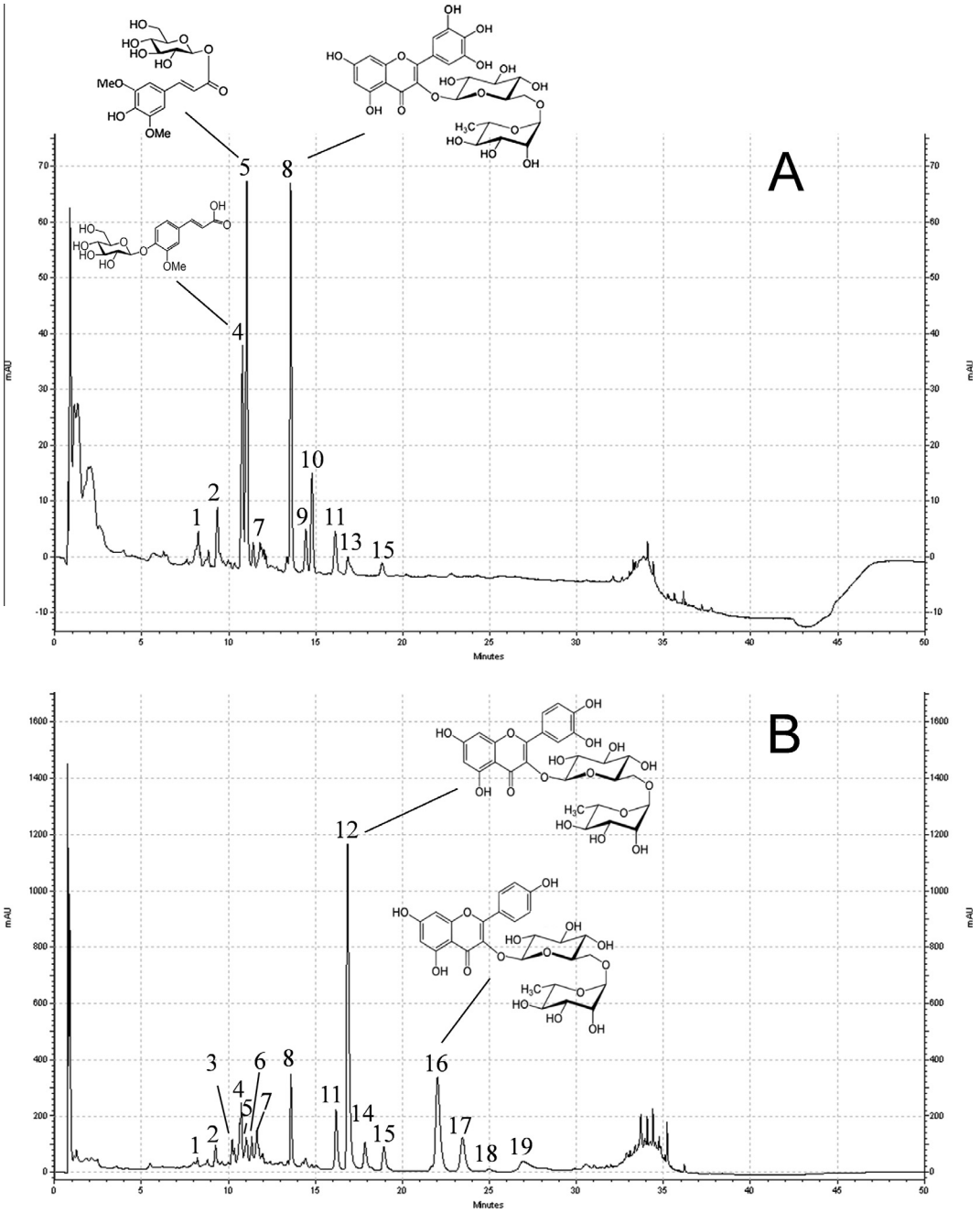

Fig. 1. Pulp (A) and peel (B) phenolic profiles of Red Yade plantain (*Musa* sp.) obtained by HPLC-DAD-HR-MS and recorded at 350 nm. Peak names: 1, caffeic acid-hexoside; 2, ferulic acid-hexoside; 3, sinapic acid-hexoside; 4, ferulic acid-dihexoside; 5, myricetin-deoxyhexose-hexoside; 6, ferulic acid; 7, sinapic acid; 8, quercetin-deoxyhexose-hexoside; 9, rutin; 10, methylmyricetin-deoxyhexose-hexoside; 11, quercetin-hexoside; 12, kaempferol-deoxyhexose-hexoside; 13, kaempferol-3-*O*-rutinoside; 14, isorhamnetin-3-*O*-rutinoside.

Table 1
Phenolic compounds determined by HPLC–ESI-HR-MS in plantain (cultivar Red Yade) pulp and peel extracts.

Peaks	RT	λ_{max}	MS[1] (*m*/*z*)			MS[2] (*m*/*z*)			Putative identification
	(Min)	(nm)	$[M-1]^-$	Molecular formula	Error (ppm)	$[M-1]^-$	Molecular formula	Error (ppm)	
Hydroxycinnamic acids									
1	8.23	287sh; 328	341.0876	$C_{15}H_{18}O_9$	−0.631	nd	nd	nd	Caffeic acid-hexoside[3]
4	10.74	291sh; 329	355.1032	$C_{16}H_{20}O_9$	−0.606	193.0506; 175.0402	$C_{10}H_{10}O_4$; $C_{10}H_8O_3$	−0.270; 0.815	Ferulic acid-hexoside[2]
5	11.02	279sh; 329	385.1137	$C_{17}H_{22}O_{10}$	−0.883	223.0611; 205.0507	$C_{11}H_{12}O_5$; $C_{11}H_{10}O_4$	−0.568; 0.185	Sinapic acid-hexoside[2]
7	11.65	282; 326	517.1567	$C_{22}H_{30}O_{14}$	0.892	193.0506; 355.1032	$C_{10}H_{10}O_4$; $C_{16}H_{20}O_9$	−0.322; −0.775	Ferulic acid-dihexoside[2]
9	14.43	281sh; 323	193.0503	$C_{10}H_{10}O_4$	−1.979	nd	nd	nd	Ferulic acid[1]
10	14.70	323	223.0611	$C_{11}H_{12}O_5$	−0.568	nd	nd	nd	Sinapic acid[1]
19	26.93	285sh; 327	379.0666	$C_{17}H_{16}O_{10}$	−1.345	185.0089	$C_7H_6O_6$	−1.195	Hydroxycinnamic acid derivative[4]
Flavonols									
3	10.23	267sh; 350	771.1986	$C_{33}H_{40}O_{21}$	−0.494	301.0352; 609.1460	$C_{15}H_{10}O_7$; $C_{27}H_{30}O_{16}$	−0.717; −0.177	Quercetin-deoxyhexose-hexoside-hexoside
8	13.59	254; 357	625.1405	$C_{27}H_{30}O_{17}$	−0.836	317.0299; 479.0830	$C_{15}H_{10}O_8$; $C_{21}H_{20}O_{13}$	−1.295; −0.279	Myricetin-deoxyhexose-hexoside[2]
11	16.20	255; 354	609.1458	$C_{27}H_{30}O_{16}$	−0.489	301.0349; 463.0880	$C_{15}H_{10}O_7$; $C_{21}H_{20}O_{12}$	−1.647; −0.408	Quercetin-deoxyhexose-hexoside[2]
12	16.85	254; 350	609.1464	$C_{27}H_{30}O_{16}$	0.414	301.0351; 463.0881	$C_{15}H_{10}O_7$; $C_{21}H_{20}O_{12}$	−0.916; −0.279	Rutin [1]
13	17.05	267sh; 357	639.1553	$C_{28}H_{32}O_{17}$	−2.194	331.0449; 315.0138	$C_{16}H_{12}O_8$; $C_{15}H_8O_8$	−3.173; −2.604	Methylmyricetin-deoxyhexose-hexoside[2]
14	15.26	254; 352	463.0884	$C_{21}H_{20}O_{12}$	0.326	301.0350	$C_{15}H_{10}O_7$	−1.149	Quercetin-hexoside[2]
15	18.95	265; 347	593.1514	$C_{27}H_{30}O_{15}$	0.399	285.0403; 447.0931	$C_{15}H_{10}O_6$; $C_{21}H_{20}O_{11}$	−0.741; −0.525	Kaempferol-deoxyhexose-hexoside[2]
16	22.02	265; 346	593.1516	$C_{27}H_{30}O_{15}$	0.703	285.0405; 447.0931	$C_{15}H_{10}O_6$; $C_{21}H_{20}O_{11}$	0.031; −0.323	Kaempferol-3-*O*-rutinoside[1]
17	23.45	254; 353	623.1616	$C_{28}H_{32}O_{16}$	−0.318	315.0508; 477.1044	$C_{16}H_{12}O_7$; $C_{22}H_{22}O_{12}$	−0.781; 1.154	Isorhamnetin-3-*O*-rutinoside[1]
18	24.98	266sh; 361	477.1039	$C_{22}H_{22}O_{12}$	−0.190	315.0508	$C_{16}H_{12}O_7$	−0.876	Isorhamnetin-hexoside
Flavanols									
6	11.35	279	289.0714	$C_{15}H_{14}O_6$	−1.112	245.0816	$C_{14}H_{14}O_4$	−1.519	Epicatechin[1]
Other									
2	9.35	272; 339	293.1236	$C_{12}H_{22}O_8$	−1.811	nd	nd	nd	Unknown

sh: shoulder.
nd, not detected.
[1] Identity was further confirmed with an authentic standard.
[2] Putative identification based on the UV spectrum, the fragmentation pattern and the molecular formulas of the parent ion and the fragments.
[3] Putative identification based on the UV spectrum and the molecular formula of the parent ion.
[4] Putative identification based on the UV spectrum.

corresponding to the elemental formulas $C_{16}H_{20}O_9$ and $C_{17}H_{22}O_{10}$, respectively. The fragment ions at *m*/*z* 193.0506 ($C_{10}H_{10}O_4$) and *m*/*z* 223.0611 ($C_{11}H_{12}O_5$) were indicative of a loss of an hexosyl unit (162 amu; $C_6H_{10}O_5$). Peaks 4 and 5 were then putatively identified as ferulic acid-hexoside and sinapic acid-hexoside, respectively. Similar compounds have previously been identified as ferulic acid-4-*O*-glucoside in berries (Schuster & Herrmann, 1985) and 1-*O*-sinapoyl-glucose in sweet pepper (Marín, Ferreres, Tomás-Barberán, & Gil, 2004). To the best of our knowledge, no report of sinapic acid-hexoside and ferulic acid-hexoside has been published before regarding *Musa* sp. fruits. The identity of other hydroxycinnamic acids was deduced from the fragmentation patterns of peaks 1, 7, 9 and 10 (Table 1). These peaks appeared to correspond to a caffeic acid-hexoside, a ferulic acid-dihexoside, ferulic acid and sinapic acid (the last two by comparison with pure standards), respectively. With a λ_{max} of 357 nm, peak 8 dominated the flavonol profile of the pulp extract (Fig. 1A). The fragmentation of its molecular ion $[M-H]^-$ at *m*/*z* 625.1405 ($C_{27}H_{30}O_{17}$) revealed the presence of a myricetin or isomer derivative (*m*/*z* 317.0299, $C_{15}H_{10}O_8$) with a substitution pattern characterized by a successive loss of a deoxyhexosyl unit (146 amu; $C_6H_{10}O_4$) and of a hexosyl unit (162 amu; $C_6H_{10}O_5$). This peak was therefore putatively identified as myricetin deoxyhexose-hexoside, however the position of the sugar linkages has to be determined. Such compounds have previously been described in many plant samples but this is the first time that it is reported in plantain banana. The fragmentation pattern analysis of peaks 11, 13 and 15 resulted in the putative identification of quercetin-deoxyhexose-hexoside, methylmyricetin-deoxyhexose-hexoside and kaempferol-deoxyhexose-hexoside or isomer, respectively (Table 1).

Flavonols were the predominant phenolic compounds in the peel, with peak 12 as a major component (Fig. 1B). The fragmentation pattern of peak 9 revealed a successive loss of a deoxyhexosyl unit (146 amu; $C_6H_{10}O_4$) and an hexosyl unit (162 amu; $C_6H_{10}O_5$) from the molecular ion $[M-H]^-$ at *m*/*z* 609.1464 ($C_{27}H_{30}O_{16}$). The resulting fragment 301.0351 ($C_{15}H_{10}O_7$) could be attributed to quercetin. The identity of this compound was further confirmed as quercetin-3-*O*-(α-L-rhamnopyranosyl-(1→6))-β-D-glucopyranose) or quercetin-3-*O*-rutinoside (rutin) by comparing its retention time and UV–Vis spectrum with those of an authentic standard. Peak 8 (myricetin deoxyhexose-hexoside already identified in pulp) and peak 16 appeared to be the second most important components in the peel. Peak 16 with a molecular ion $[M-H]^-$ *m*/*z* at 593.1516 ($C_{27}H_{30}O_{15}$) presented in its fragmentation pattern a structure related to kaempferol (*m*/*z* 285.0405, $C_{15}H_{10}O_6$) and a substitution pattern similar to the one of peaks

8 and 12. This suggested the identification of kaempferol-deoxy-hexose-hexoside, which was further confirmed as kaempferol-3-*O*-rutinoside by comparison with an authentic standard. The same analytical approach as above led to the proposed identification of peaks, 3, 4, 5, 11, 14, 15, 17 and 18 as quercetin-deoxy-hexose-hexoside-hexoside, ferulic acid-hexoside, sinapic acid-hexoside, quercetin deoxy-hexose-hexoside, quercetin-hexoside, kaempferol-deoxy-hexose-hexoside, isorhamnetin-3-*O*-rutinoside (further confirmed with a pure standard) and isorhamnetin-hexoside, respectively. Epicatechin (peak 6) was identified by comparison with a pure standard.

3.2. Contents of the major phenolic compounds identified in diverse plantain cultivars, hybrids and dessert bananas pulps and peels

Our results show that there was a strong variability between genotypes in terms of pulp ferulic acid-hexoside (Fig. 2). The highest content was obtained for the genotype Mbeta 1 with a mean value of 85.10 ± 13.52 μg/g DW, the genotype Big Ebanga having the second most important content (59.97 ± 14.74 μg/g DW). The amounts found in dessert bananas were in the range of those of plantain bananas. The hybrid harvested in Njombe had a very low content. Interestingly, this compound was not detected in the hybrid harvested in Bansoa. Banana pulp has been reported to be a rich source of ferulic acid (Zhao & Moghadasian, 2008) but their glycosylated forms have never been reported. Pulp sinapic acid-hexoside was, in most cultivars, lower than ferulic acid-hexoside and varied between 2.12 ± 0.23 μg/g DW (for the hybrid F568 Njombe) and 7.67 ± 1.57 μg/g DW (for the plantain cultivar Essang). Myricetin-deoxyhexose-hexoside was present to a large extent in all banana genotype pulps with low variability when compared to ferulic acid-hexoside. The contents found ranged from 35.12 ± 3.97 μg/g DW (for the plantain banana Mbouroukou N° 1) to 57.88 ± 8.75 μg/g DW (for the dessert banana Grand Nain). When compared to berry fruits, known as a rich source of myricetin-rutinoside, these values are lower than those reported in blackcurrants (14.7 mg/kg FW or ~79 μg/g DW) and higher than those reported in elderberry (3.7 mg/kg FW or ~19.7 μg/g DW) (Mikulic-Petkovsek, Slatnar, Stampar, & Veberic, 2012).

As shown in Fig. 3, rutin dominated the peel flavonol profile of plantain cultivars, as well as of the dessert banana Gros Michel. In contrast, rutin had lower values in the hybrids, and was not detected in the dessert banana Grand Nain. The rutin content of Gros Michel peel (494.43 ± 153.71 μg/g DW) was similar to those of plantain peels and in agreement with those reported in the peel of dessert bananas from the Philippines (550–700 μg/g DW, adapted from Kanazawa and Sakakibara (2000)). The highest value (122 ± 8.5 μg/g DW) reported for potato peel by Deußer, Guignard, Hoffmann, and Evers (2012) appears to be lower than those found in plantain peels. Furthermore, rutin contents of plantain and dessert banana peels, except Grand Nain, are in the range of those reported for buckwheat flour (218 and 792 μg/g DW), which is one of the most important known food source of rutin (Kreft, Fabjan, & Yasumoto, 2006; Tian, Li, & Patil, 2002). Myricetin-deoxyhexose-hexoside content was quantified at 125.32 ± 17.18 μg/g DW in Grand Nain peel, which, along with Gros Michel peel (172.28 ± 12.38 μg/g DW), had the highest mean values among the banana genotypes under investigation. Regarding the two samples of the hybrid F568, myricetin-deoxyhexose-hexoside was found in considerable amount in the sample from Njombe (77.56 ± 5.12 μg/g DW) whereas it was not detected in the sample from Bansoa. According to the pedo-climatic data, Njombe has a rich soil, a mean temperature and an annual rainfall more favourable for the growth of plantain bananas than Bansoa. This could have contributed to the difference observed in the phenolic profile, but further investigations with many other cultivars are needed to confirm these results. However, Jaakola and Hohtola (2010) reported that the temperature affects flavonoid content of plants, as flavonol contents were lower in strawberries grown at 18 °C, than in strawberries grown at 30 °C. Unlike in plantains, the peel kaempferol-3-*O*-rutinoside was predominant in the hybrids. Its content in plantain peel was higher than the highest content of potato peel (28.2 ± 3.2 μg/g DW) reported by Deußer et al. (2012).

The major phenolic compounds found in plantain bananas have been reported, in the literature, to exhibit numerous biological effects. Sinapic and ferulic acid-glycoside esters were found to be efficient antioxidants (Kylli et al., 2008). It has been reported that phenolic acids and flavonol glucuronides and sulfates, which are among the plasma metabolites of the phenolic compounds found

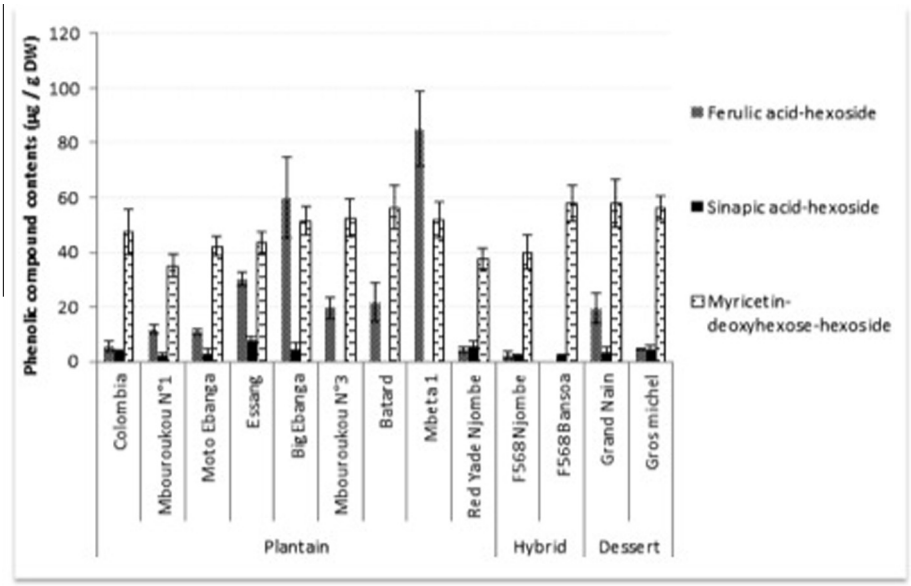

Fig. 2. Contents in ferulic acid-hexoside, sinapic acid-hexoside and myricetin deoxyhexose-hexoside of the pulps of *Musa* sp. genotypes resulting from the analyses of three biological replicates ($n = 3$) each extracted in duplicate. Ferulic acid-hexoside and sinapic acid-hexoside are expressed in ferulic acid equivalents, myricetin-deoxyhexose-hexoside is expressed in myricetin equivalents.

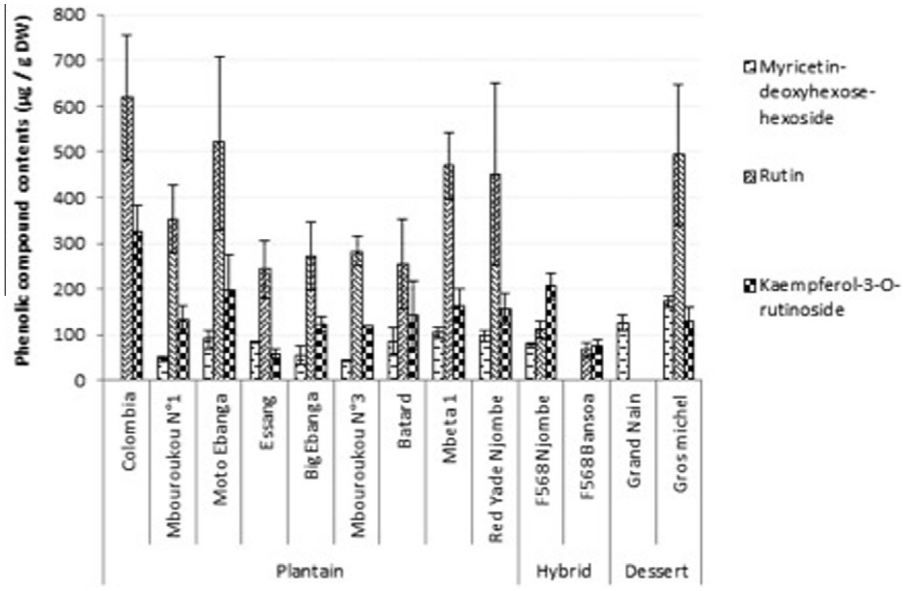

Fig. 3. Contents in myricetin-deoxyhexose-hexoside, rutin and kaempferol-deoxyhexose-hexoside of the peels of *Musa* sp. genotypes resulting from the analyses of three biological replicates ($n = 3$) each extracted in duplicate. Myricetin-deoxyhexose-hexoside is expressed in myricetin equivalents.

in this study, might release their free forms at target tissues where the aglycones act as the active molecules (Manach et al., 2004). Furthermore, *in vivo* studies showed that ferulic acid and sinapic acid administered by intraperitoneal injection to mice were able to exert protective effects against traumatic brain injury (Zhang et al., 2007), atherosclerosis (Kwon et al., 2010), and liver toxicity (Pari & Mohamed Jalaludeen, 2011). Numerous *in vivo* and *in vitro* studies have shown that myricetin has potent antioxidant properties. It is notably able to reduce the by-products of the lipid peroxidation and to stimulate the expression of antioxidant enzymes (i.e. catalase, glutathione peroxidase) in induced colon cancer (Nirmala & Ramanathan, 2011). *In vitro* anti-cancer properties have also been reported for myricetin as it may induce apoptosis in human hepatocellular carcinoma cells (Zhang, Ling, Yu, & Ji, 2010). Rutin, the predominant phenolic compound in plantain peel, has been marketed as nutraceutical and is well known for its ability to strengthen blood vessel walls (Sun & Ho, 2005). The wound healing effect of a hydrogel containing 0.025% of rutin after cutaneous application was successfully investigated by Almeida et al. (2012). Similarly, a gel composed of 4% of banana peel was found to repair surgical wound in rats (Atzingen et al., 2013). Rutin has also shown an anti-diabetic effect by regulating glucose metabolism and improving antioxidant status in diabetic rats (Krishna et al., 2005). Taken together, plantain peel flavonols could have contributed to the antioxidative effect of plantain peel methanolic extract against the oxidation of refined soybean oil (Arawande, Amoo, & Lajide, 2010). Moreover, our study showed that plantain peel contains the glycosylated form of the flavonol myricetin that have been successfully tested for its protective effect against linseed oil oxidation (Michotte et al., 2011). Our study, together with the cited studies, suggest a contribution of those compounds in the reported health-promoting effects of plantain banana peel and show a way of using plantain banana in food and pharmaceutical industries.

In Europe, plantain peel could be proposed as a novel food ingredient, and also as a plant ingredient for novel medicine formulation. In the first case, criteria set out in the European regulation number 258/97 will have to be fulfilled. This requires the development of efficient procedures to avoid or remove all hazardous related to the agricultural practices and the safety assessments to provide sufficient evidences that the proposed conditions of uses are harmless. In the second case, sufficient proofs of the safety and the efficacy of the proposed medicinal product for human or veterinary uses will have to be submitted to the European Medicine Agency (EMA).

3.3. Contribution of the major phenolic compounds to the Musa sp. fruit diversity

To evaluate the contribution of the major phenolic compounds to cultivar diversity, a principal component analysis was performed on all the data (reported in the Supplementary Tables 1 and 2). Fig. 4 presents the loading plot of phenolic compounds (A) and the scatterplot of dessert, hybrids and plantain banana cultivars (B). The two first principal components (PC1 and PC2) explained most of the variation and accounted for 58.3% of the total variation.

The directions of the phenolic vectors (Fig. 4A) show that the pulp ferulic acid-hexoside was positively correlated to the other pulp hydroxycinnamic acids excluding sinapic acid glycosides, suggesting that the control of the sinapic acid glycosides biosynthetic pathway differed from those of the other pulp hydroxycinnamic acids. Pulp and peel myricetin-deoxyhexose-hexoside appeared to be positively correlated. Peel rutin was positively correlated to the other peel flavonols. A simultaneous analysis of the directions of the phenolic compound vectors (Fig. 4A) and the position of cultivars (Fig. 4B) reveals that peel rutin contributed to the separation of cultivar groups along the PC1 axis. With high levels of pulp and peel myricetin-deoxyhexose-hexoside, dessert bananas were separated from the other groups. Pulp ferulic acid-hexoside, caffeic acid-hexoside, ferulic acid and ferulic acid-dihexoside, together with peel kaempferol rutinoside, kaempferol dehoxyhexose-hexoside and sinapic acid-hexoside were the most involved in plantain cultivar diversity. They strongly contributed to the separation of plantain banana cultivars on the two diagonals of the plane formed by the PC1 and PC2 axes. Since it has been reported that molecular markers hardly enable the differentiation between plantain cultivars (Noyer, Causse, Tomekpe, Bouet, & Baurens, 2005), the search for other markers, such as chemical markers, is of a great interest.

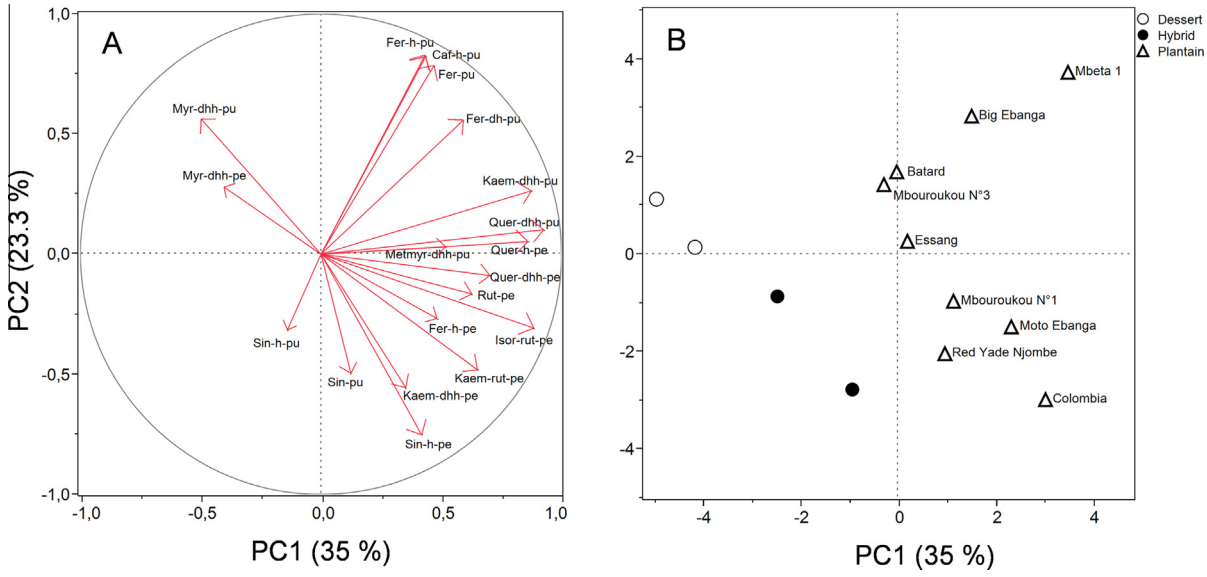

Fig. 4. Principal component analysis resulting from the phenolic compound profiling of plantain pulp and peel. (A) Loading plot of the phenolic attributes and (B) Scatter plot of plantain varieties. Pu (pulp), pe (peel), Caf-h (caffeic acid-hexoside), Fer-h (ferulic acid-hexoside), Fer-dh (ferulic acid-dihexoside), Sin-h (sinapic acid-hexoside), Sin (sinapic acid), Myr-dhh (myricetin-deoxyhexose-hexoside), Fer (ferulic acid), Quer-dhh (quercetin-deoxyhexose-hexoside), Rut (rutin), Metmyr-dhh (methylmyricetin-deoxyhexose-hexoside), Quer-h (quercetin-hexoside), Kaem-dhh (kaempferol-deoxyhexose-hexoside), Kaem-rut (kaempferol-3-*O*-rutinoside), Isor-rut (isorhamnetin-3-*O*-rutinoside).

The use of hydroxycinnamic acids, which have been shown to largely contribute to the plantain cultivar diversity, could be relevant to highlight the polymorphisms within plantains. No distinction between French and Horn plantain bananas was observed, meaning that the evolution of the male bud had no remarkable effect on the phenolic content of plantain bananas.

4. Conclusions

This study attempts to identify the phenolic compounds of plantain cultivars and thereby better understand their health-promoting effects. The results obtained show that the pulp phenolic profile was dominated by hydroxycinnamic acids, whereas the peel phenolic profile was dominated by flavonols. These identified compounds could contribute to the reported health-promoting properties of plantain. Furthermore, plantain, as well as some dessert banana peels, could be exploited in food and pharmaceutical industries for their contents in flavonols and their high content in rutin.

The identified phenolic compounds also contributed to the diversity of the *Musa* sp. fruit groups and plantain cultivars, but did not enable plantain groups to stand out. These results arouse further investigations on a whole plantain banana collection, like the one of the CARBAP, in which the identified hydroxycinnamic acids could be used as polymorphism markers. However, additional chemistry work is needed to identify the exact structure of certain pulp and peel flavonols for a subsequent use in plantain chemotaxonomy. A hypothesis according to which the environment could contribute in the selection of phenolic biosynthetic pathways seemed to emerge from this work. This calls for further investigations on the impact of the pedo-climatic conditions on pulp and peel phenolic profiles.

The fruits analysed in this study were ripe and raw. Knowing that there are many ripening stages that can affect the contents, and that after the cooking processes the quantities ingested will not be the same, it would be worth undertaking studies on the impact of the ripening stages and the cooking processes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.06.095.

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